**Synopsis of the Ph.D., Thesis entitled**

# Variability Studies on *Invitro Callus* Formation and Plantlet Regeneration In Certain Mulberry Varieties (Morus Spp.) Moraceae".

## SYNOPSIS

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## Guide

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# INTRODUCTION

Mulberry is cultivated in different parts of the world largely for foliage, used for feeding silkworms *(Bombyx mori.L)* for the production of silk. But depending on the location, it is also appreciated for its delicious fruit and medicinal properties, as animal feed, and for landscaping. Mulberry is also utilized traditionally as a feed in mixed forge, diets for ruminants. The term morus comes from Latin mora meaning delay, probably because of very slow development of its buds. Mulberry trees are either dioecious or monoecious. and sometimes change from one sex to another. The flowers held on short, green, pendulous, non-descript catkins, are wind pollinated, or do not need to be pollinated at all. Some cultivars bear fruit without any pollination mulberries in California are an example. Major species of mulberry are, Morus alba (White), M.Nigra (black) and M.rubra (red).Also, hybrids exist between M.alba and M.rubra. Other related spcies are Korean mulberry(Morus australis), Himalayan mulberry (M. laevigata) etc.

The species vary greatly in longevity. Red mulberry trees rarely live more than 75 years, while black mulberries have been known to bear fruit for hundreds of years. As regards growth, fast growing mulberry trees can grow up to 10 ft in one year, and are variable in size according to the species- M. alba (up to 80 ft), M. rubra (70 ft) and the smallest being M. nigra (30 ft). Morus alba is cultivated through the world wherever silkworms are raised. In recent years, mulberry has been re-evaluated due to its functional characteristics and is being utilized for various puiposes. Anantha cultivar is known for its high level leaf of yield, 60-70 tones/ha, and curd protein 25-35% and leaf moisture is 80-85%, total minerals 20-80% where compared to M5 which is heavy leaf yield of about 22-23 tones/ha. Crude protein 18-27%, leaf

moisture 75-82% total minerals 18-73% and carbohydrates 12.20%.Further seeds are not available in Anantha variety for seedling propagation. Hence, it is essential to develop a-strandized protocol to propagate and supply the Anantha cultivar plantlets through micro propagation techniques. Further, it is also standardized protocol to propagation of the variety $M_5$, $S_{36}$, $V_I$ and Anantha and compared.

## Aim of the study

The present investigation is having worth scientific utility and aimed: To standardize protocols for the initiation of callus mainly from explants of different varieties of mulberry varities (*Morus Spp.*). The family *Moraceae* is one of the most responsive and diverse plant groups in the context of the application of tissue culture techniques. It has long been recognized that cells, tissues and organs from members of this family undergo morphogenesis and *invitro* plant regeneration easily. However tissue culture of certain mulberry varieties (*Morus Spp.*) are lagged behind, most likely due to the lack of the success in early attempts to regenerate plants from different explant cultures. Most works on *invitro* techniques in mulberry are concerned with direct organogenesis. Detailed investigation on variability aspects of callus formation, shoot formation, rooting and biochemical changes during their regeneration and multiplication in Indian varieties is very meager. Hence, variability aspects of callus formation, shoot formation, rooting and biochemical studies have been studied in the mulberry, *Morus Spp.* Moraceae, with reference to nutrient media, plant growth regulators, carbon source.

## Significance of the study

Genotypes variations in magnitude of invitro responses in several crop plants have provided significant information concerning their adaptability, callus formation, plantlet regeneration, rooting and acclimatization and biochemical changes during organogenesis/plantlet regeneration in culture media. Preliminary studies have been conducted to screen some of the promising varieties in the Rayalaseema region of Andhra Pradesh and based on these studies four mulberry varieties were selected for tissue culture. Hence, the studies on evaluation of suitable media, callus, plantlet formation, rooting and acclimatization were carried out using different media with different combinations of hormones in four mulberry varieties of *Morus Spp*. Further, biochemical studies were carried out in the regenerant tissues of four Mulberry varieties for the characterization of the active growth patterns.

## Objectives

In order to employ tissue culture techniques for the improvement of mulberry, four varieties viz. $M_{5,}$ V-1, $S_{36}$ and Anantha were chosen for the study with the following objectives.

1. Optimization of surface sterilant for the culture initiative of Mulberry varieties $M_5$, V1, $S_{36}$ and Anantha.
2. Optimization of nutrient media, plant growth regulators and carbon source for achieving initiation of callus mainly from auxiliary bud explants of four varieties of *Morus Spp.* ($M_5$,V-1 $S_{36}$ and Anantha)
3. Establishment of primary sprouts and sub sequent initiation as well as secondary sprouts of Mulberry varieties $M_5$, V1, $S_{36}$ and Anantha.
4. Extrapolations of the standardized protocol for shoot *invitro* multiplication and *invitro* rooting and subsequent acclimatization of plantlets of Mulberry varieties $M_5$, $V_1$, $S_{36}$ and Anantha.
5. To confirm the plantlet regenerations based on biochemical changes.

## CHAPTERIZATION

## CHAPTER -I: GENERAL INTRODUCTION

This chapter deals with an overview of developments in plant tissue culture which helps us to understand various morphological variations encountered during the culture of Mulberry. *In vitro* studies carried out in Mulberry so far have been discussed.

## CHAPTER - II: MATERIALS AND METHODS

Procurement of plant material, composition of various plant tissue culture media have been included in this chapter and also various techniques used during the course of this work.

## Chapter-III: Evaluation of suitable media

Between the two different media such as MS and B5 with 3mg/l BAP + 0.05 mg NAA tested for Morus Spp. varities $M_5$, $S_{36}$, $V_I$ and Anantha, MS medium was found to be best basal medium for shoot induction when compared to the B5 media. Further, the auxiliary buds exhibited high percentage of callus formation and weight of callus in all the mulberry varieties. Hence, only MS medium was used to carry out all the *invitro* experiments for plantlet regenerations. In the present study different mature

explants such as petiole auxiliary bud and shoot tips of Morus Spp. $M_5$, $S_{36}$, $V_I$ and Anantha were screened to evaluate best explant on different basal media containing BAP 3mgl$^{-1}$ + 0.05 mg NAA. Among the various explants tested, only those of auxiliary buds and shoot tip explants showed positive morphogenetic response. In $M_5$, $S_{36}$, $V_1$ and Anantha species auxiliary bud explants were more effective for proliferation of shoot than shoot tip explants, whereas petiole failed to initiate shoots.

## Chapter- IV: Callus formation

In our present study highest callus initiation was observed at 2mg/l 2, 4-D concentration. Mulberry varieties $M_5$, $S_{36}$, $V_1$ and Anantha were cultured on MS media, supplemented with 2,4-D 2 mg$^{-1}$. MS medium containing 2 mg/1$^{-1}$ 2,4-D produced maximum fresh and dry weight of callus in 20 days. Highest callus initiation was observed at the concentration of 2 mg$^{-1}$ of 2, 4. D. Thus the present study provides a scope of rapid callusing of $M_5$, $V_1$, $S_{36}$ and Anantha on MS + 2, 4. D (2,4-Dichloro phenoxy acetic acid).

## Chapter- V: Shoot multiplication

Auxiliary bud explants were cultured on basal medium (MS) supplemented with various cytokinins, auxins and antioxidants either alone or in combination. BAP, Kinetin and 2-ip were used to select best cytokine for shoot proliferation. BAP was found to be effective than 2-ip and kinetin. BAP of 2mg$^{-1}$ was found to be optimum concentration in four mulberry varieties. To improve further shoot multiplication rate of various combinations of three cytokinines + auxin were used. Highest shoot multiplication from the auxiliary bud explants of Morus Spp varieties were observed on MS medium with BAP 2 mg$^{-1}$ +NAA 1mg$^{-1}$.

## Chapter-VI: *In vitro* rooting and acclimatization

*In vitro* grown shoots from nodal cultures having 2-3 nodes were excised and inoculated on half strength MS medium fortified with various auxins such as NAA, IAA and IBA in all four mulberry varieties. IBA was effective for *in vitro* rooting followed by NAA and IAA. *In vitro* plantlets with well developed roots were transferred to pots containing vermiculate and plantlets were subsequently acclimatized.

## Chapter-VII: Biochemical Studies

Since the building up and breaking down of protoplasm of regenerants is concerned with certain metabolites and certain enzyme activities, some biochemical studies were carried to know the plantlet regeneration and growth pattern of Mulberry varieties such as M5, S36, V1 and Anantha, during their multiplication and regeneration into shoots and plantlets. The changes in the metabolites such as starch, reducing sugars and total sugar content indicate that the accumulation of these metabolites till day '15' seems to reflect the high energy requirement of the organogenic processes, also this accumulation of starch and sugars play an important role as osmotic agents and further decrease in the levels is of much significance with associated visible manifestation of organogenesis. Increased activity of these hydrolytic enzymes such as amylases and acid and alkaline phosphatase enzymes during the present investigation indicated that degradation of different compounds proceeded in the regenerating tissues and this was concurrent with the high synthetic activity that occurs during organogenesis. Changes in the activities of soluble acid and wall bound invertase activities indicate the peak activities of the enzyme exhibits most rapid cell expansion in the regerants. The increased activities of nitrate reductase (s) and decreased activities of GDH isoforms confirm the active multiplication of explants without any sign of vitrification.

On the whole *invitro* proliferated shoots were multiplied rapidly by culture of auxiliary nodal explants on MS medium. Media containing 3 $mg^{-l}$ 2-4 D, produced maximum fresh and dry weight of callus. Highest shoot multiplication was obtained on MS media with BAP 2 $mg^{-l}$ + NAA 1$mg^{-l}$. Highest frequency of rooting was obtained from shoots cultured on MS medium with 0.2 $mg^{-l}$ IBA. Biochemical studies exhibits an accumulation of total and reducing sugars, starch and total soluble proteins and hydrolytic enzymes confirm the active proliferation of explants, later regenerated into plantlets. Increased activities of nitrate reductase (s), and decreased activities of GDH isoforms and it confirms the active multiplication of regenerants without any sign of vitrification. The invitro studies established in this study is an effective means for large scale micropropagation of commercially useful mulberry varieties. Though there was a similar trend in the entire mulberry varieties in the above said parameters, there were significant percent variations among the mulberry varieties in the same trends.